SF 298 MASTER COPY KEEP THIS COPY FOR REPRODUCTION PURPOSES

REPORT DOCUMENTATION PAGE

Form Approved
OMB NO. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE

3. REPORT TYPE AND DATES COVERED
Final Report - 5/10/93 - 5/9/96

4. TITLE AND SUBTITLE
Genetic Engineering of Polymers Containing Non-Natural Amino Acids

5. FUNDING NUMBERS
DAAH04-93-G0217

6. AUTHOR(S)
David A. Tirrell

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)
Department of Polymer Science and Engineering
University of Massachusetts
Amherst, MA 01003

8. PERFORMING ORGANIZATION REPORT NUMBER
N/A

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
ARO 31087.1-LS

11. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12 b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The goal of this research program has been to establish strategies for producing protein-based materials that contain amino acids not normally found in natural proteins. Efforts have been focused on amino acids with fluorinated, electroactive or conformationally constrained side chains, and have allowed us to prepare protein-based polymers with unique properties, including low surface energies and sensitivity to electrochemical signals. These developments have expanded in a substantial way the range of materials properties accessible via chemical or biological synthesis of polypeptides and artificial proteins.

19960909 130

14. SUBJECT TERMS
genetic engineering, polymers, amino acids, proteins

15. NUMBER IF PAGES
5

16. PRICE CODE

17. SECURITY CLASSIFICATION OR REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. 239-18
298-102

Genetic Engineering of Polymers Containing Non-Natural Amino Acids

FINAL PROGRESS REPORT

David A. Tirrell
Principal Investigator

June 1996

U.S. Army Research Office
DAAH04-93-G-0217

University of Massachusetts Amherst

Statement of the Problem

The goal of this research program has been to establish strategies for producing protein-based materials that contain amino acids not normally found in natural proteins. Efforts have been focused on amino acids with fluorinated, electroactive, or conformationally constrained side chains, and have allowed us to prepare protein-based polymers with unique properties, including low surface energies and sensitivity to electrochemical signals. These developments have expanded in a substantial way the range of materials properties accessible via chemical or biological synthesis of polypeptides and artificial proteins.

Summary of Results

Previous progress reports have discussed our results on *p*-fluorophenylalanine[1], trifluoroleucine[2], azetidine-2-carboxylic acid[3], thiaproline, dehydroproline[4], and 3-thienylalanine[5]. All of these amino acid analogues have been successfully incorporated into artificial proteins expressed in bacterial hosts, via routes that exploit the promiscuity of the normal protein synthetic apparatus of the bacterial cell. During the most recent funding period, primary attention has been given to two new directions that are complementary to the first: i). chemical synthesis and structural analysis of poly(α,L-glutamate)s with long fluoroalkyl side chains, and ii). development of new methods for site-directed incorporation of non-natural amino acids in *Escherichia coli.*

Poly(α,L-glutamate)s with fluoroalkyl side chains. Fluorinated polymers exhibit special properties, including stability at high temperatures, toughness and flexibility at very low temperatures, nonadhesiveness, insolubility, chemical inertness, and, in some cases, biocompatibility. Incorporation of fluorinated amino acids has been proposed as a means to impart some of these properties to polypeptides. In work reported earlier this year in *Macromolecules*[6], poly(α,L-glutamate)s carrying C_8, C_{10}, C_{12} fluorinated side chains were synthesized by polymerization of the corresponding amino acid N-carboxyanhydrides. Contact angle measurements with water were used to assess the effects of fluorination on the surface energies of films of the resulting polypeptides. The wettability of the polymers was found to decrease with increasing fluorine content, as expected. A remarkably high contact angle (121°) was measured for the homopolymer carrying C_{12} fluorinated side chains, indicating a surface consisting of closely packed trifluoromethyl groups. Side chain crystallization, consistent with the formation of such a surface, was suggested by the results of x-ray diffraction and calorimetric measurements.

Site-directed incorporation of non-natural amino acids in E.coli. In this phase of our program we are developing *E. coli* expression strains for the site-directed incorporation of non-natural amino acids.

Our approach entails encoding the position of the non-natural amino acid in the gene of choice by the "blank" amber stop codon (TAG); this stop codon is deciphered in translation by a yeast amber suppressor tRNA, which in turn is charged by its co-expressed cognate yeast tRNA synthetase (a so-called 21st pair) with an analogue provided in the growth medium. The long-term goal of this work is the creation of mutant yeast tRNA synthetases which can charge useful non-natural amino acids with high specificity to their cognate suppressor tRNAs. So far, we have identified the yeast seryl-tRNA synthetase (SRS) and the $tRNA^{ser}_{amber}$ as a suitable 21st pair for our purposes. We are currently identifying active-site residues in the yeast SRS that make essential contacts to the serine substrate side chain. Such residues will be prime targets in our effort to change substrate specificity through protein engineering.

Our shorter term goal is a direct application of the 21st pair approach, namely the site-directed incorporation of p-fluorophenylalanine (pFPhe) as a useful ^{19}F-NMR probe. The analogue pFPhe is a cytotoxic substrate for *E. coli*, but a pFphe resistant mutant strain isolated a long time ago rejects this non-natural amino acid, owing to increased substrate specificity of its phenylalanyl-tRNA synthetase (FRS). We have equipped this resistant strain with a yeast $tRNA^{Phe}_{amber}$ and yeast FRS. Co-expression of this 21st pair in the absence of analogue results in incorporation of about 95% Phe at the programmed position in the marker protein dihydrofolate reductase (DHFR), demonstrating that the yeast $tRNA^{Phe}_{amber}$ is not mischarged by the other host synthetases. Preliminary data show that when an 8-fold excess of pFPhe over Phe is present in the growth medium, the programmed position in DHFR now contains about 70% pFPhe and about 30% Phe as determined by peptide sequencing. Even though complete substitution of Phe by pFPhe cannot be achieved in this system at this point, unlabeled species will be silent in the NMR measurement and will not complicate data aquisition with the labeled fraction of the sample.

Future experiments are designed to corroborate the preliminary finding, to show that only the programmed position contains the analogue, and to increase the expression yield about 10-fold from the current 0.8 mg/L of culture at $OD_{600} = 1.0$. We also have indirect genetic evidence that suggests that p-chlorophenylalanine might be incorporated by the 21st yeast pair as well.

Our current experiments are directed toward *site-directed* insertion of non-natural amino acids into proteins. The synthetases and strategies developed here, however, can also be used, with some modification, for *uniform* labeling of protein-based polymer materials with non-natural amino acids. We intend to begin exploration of this possibility during the coming months, pending continued ARO support of this work.

Publications

The papers and manuscripts resulting from this program are listed below.

1. Yoshikawa, E., Fournier, M. J., Mason, T. L., & Tirrell, D. A. (1994). Genetically Engineered Fluoropolymers. Synthesis of a Repetitive Polypeptide Containing p-Fluorophenylalanine Residues. Macromolecules, 27, 5471-5475.
2. Kothakota, S., Atkins, E. D. T., Mason, T. L., Tirrell, D. A., & Fournier, M. J. Genetically Engineered Fluoropolymers with Reduced Surface Energy, in preparation.
3. Deming, T. J., Fournier, M. J., Mason, T. L., & Tirrell, D. A. (1996). Structural Modification of a Periodic Polypeptide through Biosynthetic Replacement of Proline with Azetidine-2-carboxylic Acid. Macromolecules, 29, 1442-1444.
4. Deming, T. J., Fournier, M. J., Mason, T. L., & Tirrell, D. A. Biosynthetic Incorporation and Selective Chemical Modification of Functionality in Genetically Engineered Polymers, submitted for publication.
5. Dessipri, E., & Tirrell, D. A. (1994). Trifluoroalanine N-Carboxyanhydride: A Reactive Intermediate for the Synthesis of Low Surface Energy Polypeptides. Macromolecules, 27, 5463.
6. Dessipri, E., Tirrell, D. A., & Atkins, E. D. T. (1996). Fluorinated Poly(a,L-glutamate)s. Macromolecules, 29, 3545-3551.
7. Kothakota, S., Mason, T. L., Fournier, M. J., & Tirrell, D. A. (1995). Biosynthesis of a Periodic Protein Containing 3-Thienylalanine: A Step Toward Genetically Engineered Conducting Polymers. Journal of the American Chemical Society, 117, 536-537.
8. Kothakota, S., Yoshikawa, E., Murphy, O. J., Mason, T. L., Fournier, M. J., & Tirrell, D. A. (1995). A Simple Assay for Screening Translational Activity of Non-Natural Amino Acids. Implications for Polymer Synthesis on Messenger RNA Templates. Journal of Polymer Science, Part A: Polymer Chemistry, 33, 1267.
9. Tirrell, J. G., Fournier, M. J., Mason, T. L., & Tirrell, D. A. (1994). Biomolecular Materials. Chemical & Engineering News, (December 19, 40.
10. Fournier, M. J., Mason, T. L., & Tirrell, D. A. (1995). Role of Molecular Genetics in Polymer Materials Science. New York: VCH Publishers.

Scientific Personnel Supported By This Project

Srinivas Kothakota, Ph.D. 1995, Biochemistry and Molecular Biology

Eugenia Dessipri, Ph.D. 1995, Polymer Science and Engineering

Michael Yu, Ph.D. candidate Polymer Science and Engineering

Timotny Deming, Postdoctoral Fellow, Polymer Science and Engineering (now Assistant Professor of Materials, University of California, Santa Barbara)

Rolf Furter, Research Assistant Professor, Biochemistry and Molecular Biology

Report of Inventions

None to report.